REMARKS

Claims 15 and 17-22 are pending for prosecution. Claims 17-19 and 22 have been cancelled. Claims 15, 20, and 21 have been amended. New claims 29-33 have been added. Support for the amendments in claim 15 can be found on pages 11-14 and Example VI.

Applicants have removed any hyperlinks and/or other form of browser-executable codes as requested by the Examiner. Additionally, Applicants have corrected several typographical errors in the specification.

Rejection under 35 U.S.C. §102 and §103

The Examiner has rejected claims 15, 19-20 and 22 as anticipated by MacWilliams et al., claims 15, 18-22 as anticipated by Gubler et al., and claims 17 as obvious in view of MacWilliams et al. in view of Gubler et al.

MacWilliams et al and Gubler et al. are both directed to Type I restriction endonucleases. Gubler et al. describe how recognition sequences in these endonucleases may be switched by single recombination events. MacWilliams et al. describe the free assortment of subunits that arises for a Type I restriction endonuclease in a host cell such that the 3′ half or the 5′ half of the gene is sufficient to code for the enzyme specificity peptide. However, the repeat sequence on either side of the half gene results in loss of specificity.

The references are directed to subject matter that is distinct from the present claimed invention. Modular Type I restriction endonucleases are

significantly different from modular Type IIG restriction endonucleases as exemplified for reasons that include the following:

The organization of the Type I endonucleases, described in the references, differs significantly from that of the Type II endonucleases. The Type I endonuclease is encoded by a transcriptional unit encoding a specificity domain and a methylase domain (hsdM and hsdS), and a transcriptional unit encoding a restriction domain (hsdR).

> All the genetic loci coding for type I R-M systems identified so far are organized in two transcriptional units, one containing the genes hsdM and hsdS [M-S], the other containing hsdR [R] only. (Gubler et al. p. 233)

Indeed, Type I specificity polypeptide (HsdS) must interact first with a methylase polypeptide (HsdM) which in turn must interact with a separate cleavage polypeptide (HsdR) before the Type I restriction endonuclease can be active.

In contrast, the Type II systems Applicants describe, such as CspCI, [R-M] forms one transcriptional unit (gene) while [S] forms the second transcriptional unit (gene). In the Type II system, the cleavage domain and the methylase domain are tethered in a single polypeptide. Thus, the modified specificity domain need only interact with a single polypeptide having methylation and cleavage functions for the restriction endonuclease to be active.

Additionally, in contrast to the Type II endonucleases, Type I endonucleases cleave randomly at variable distances from the specificity

domain. It is thought that this is the result of spooling of the DNA through the restriction/modification domain.

> The process leading to cleavage by type I restriction enzymes at seemingly random sites up to several thousand base pairs (bp) distant from their recognition sequence has been explained by ATP-stimulated translocation of the enzymes along the DNA (Gubler et al. pg 233)

In contrast, the Type IIG restriction endonucleases cleave at a specific cleavage site on DNA at affixed distance from the recognition sequence. The Type II restriction endonucleases generated by the methods disclosed herein thus act on DNA in a fundamentally different way than the Type I systems described by the cited references.

Other differences include the established dimeric structure of the specificity domain of Type I restriction endonucleases which have been shown to have two variable regions that recognize specific base sequences, separated by an extended duplex alpha helix region.

> ... we postulate that the *Eco*R124I and *Eco*DXXI R-M systems exhibit a high degree of structural conservation.... (Gubler et al. p. 234 second col)

> ... we decided to recombine the *hsdS* genes of *Eco*R124I and *Eco*DXXI *in vitro* at specific positions....tested in phage infection assays ....(p. 235)

In contrast to the Type I restriction endonucleases, the group of enzymes exemplified by CspCI are separated by a shorter non-homogeneous linker region (only 15A angstroms) (see page 43, line 27 of the application).

Unlike for Type I restriction endonucleases, homologous recombination apparently does not readily happen for Type IIG restriction endonucleases *in vivo* as evidenced by the lack of varients of any particular endonucleases.

Despite the significant differences between Type I and Type II restriction endonucleases, Applicants nonetheless determined that the specificity region of Type II endonucleases could be modified in various ways to alter the specificity of the Type II restriction endonucleases using *in vitro* molecular biology techniques instead of *in vivo* homologous recombination.

The Examiner is requested to note that despite publication of the cited art in 1992 and 1996 for Type I restriction endonucleases, almost 10 years before the present application was filed, there has been no report prior to the present application of attempting to modify the specificity of a Type IIG endonuclease by modifying the specificity domain.

Applicants respectfully assert that for the reasons provided above, the present claims are novel and non-obvious and that the cited art directed to Type I restriction endonucleases are directed to significantly different subject matter. Therefore the rejection should be reversed.

Claim Objections

Claim 15: "obtaining the restriction endonuclease": Antecedent basis has been provided by amending claim 15.

Claim 20: "specificity module" has been changed to "specificity subunit".

Rejection under 35 U.S.C. §112

Claim 20 was rejected as indefinite because it depended in part on a cancelled claim 16. This defect has been corrected.

CONCLUSION

Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension and authorize that the extension fee of $555 be charged to Deposit Account No. 14-0740. Please charge any deficiencies to the same Account.

Respectfully submitted,

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